

Nutritional composition of some selected wild mangrove fruits of Sundarbans

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Present study consists of the chemical composition and phenolic compound identification of some common wild fruits in Sundarbans mangrove. Five mangrove fruits exhibited high percentage of carbohydrates (27.25-62.9), protein (1.2-45.48), lipid (1.75-4.31) and ascorbic acid (0.013-0.032%). Occurrence of 1.70-12.35% phenolic compounds in fruits reflects their higher antioxidant capacity. ESI-MS were used to identify the occurrence of phenolic compounds in *Heriteira fomes* and *Bruguiera parviflora* and results revealed that catechin was the commonly occurring major flavonol. Hydroxycinnamic acid derivatives (p-coumarylhexose-4-hexoside) and quinic acid were also identified as minor components. Higher range of nutritive value (271.4- 330.16 kcal/100g) and comparative study with some commercial fruits together suggested that wild mangrove fruits are edible like conventional popular fruits.

[Key words: Chemical composition, antioxidant capacity, mangrove, fruit, India]

Introduction

Sundarban is a unique bioclimatic zone in land ocean boundaries of Bay of Bengal and is the largest delta on the globe. Sundarban consisted of several numbers of discrete islands and Lothian Island is one of such island covering a total area of 38 km². It is situated at the confluence of Saptamukhi River and Bay of Bengal. Lothian Island having approximate geographical position at longitude 88°19' and latitude 20°50' has been notified as wild life sanctuary in 1976. The island has been rich in mangrove floral diversity and species like *Heriteira fomes* (local name Sundari) are thinly distributed where as other species like *Cerriops decandra* (local name Goran) and *Bruguiera gynorrhiza* (local name Kakra) are found scatter all over the island.

The livelihood of nearly 2 million people in this part of the world is linked with the non-agricultural sources, which mainly include fishing and allied activities like honey and wax collection from the rivers and creeks as one of the major sources of income¹. Plant parts like stem, branches are also used for timber value and

fuelwood properties by local inhabitants. Present study will help Sundarban dwellers to select some mangrove fruits for dietary purpose.

Materials and Method

Fifteen fruits were picked manually from five different mangrove plant species at different sites of the Lothian Island during July 2011 and September, 2011. After the collection the sample were cleaned and carried to the laboratory and stored at 20 °C for freeze drying. The dried tissues were ground to a particle size of 200um and used for chemical analysis². All the treatments were replicated thrice.

Two types of mangrove fruit samples (*Heriteira fomes* and *Bruguiera parviflora*) were milled in liquid nitrogen, and three replicates (2 g each) were withdrawn for extraction. Each replicate were extracted with acetone (10 ml) by sonication for 10 min. After centrifugation the supernatant was collected and the insoluble plant material was re-extracted twice using 70% acetone (10 ml). Acetone was removed from pooled extracts in nitrogen atmosphere (-18°C). The volume of the extract was made up to 15ml by water.

Total phenolic compound in the fruit extract was determined using Folin Ciocalteu's reagent and sodium carbonate. Absorbance was measured at 765 nm using gallic acid^{3,4} as a standard.

High performance liquid chromatography (HPLC) coupled with mass spectrometry (LC-MS) have been extensively used for the isolation and characterization of phenolic compounds⁵. While³ characterized phenolic profiles of yerba maté and green tea by direct infusion electrospray using the complementary information from the DAD, ESI-MS in negative and positive modes. Some researchers⁶ identified sixteen compounds, mainly flavonoid glycosides and flavonoid aglycons in argon fruit pulp. Variation in antioxidant activity in association with polyphenolic compounds occurring in cactus pear fruits are reported⁷ in different environmental conditions.

Phenolic compounds in *Heriteira fomes* and *Bruguiera parviflora* fruits were identified by both positive and negative ion mode electrospray ionization mass spectrometry (ESI-MS) fingerprinting (scan range 50-3000 m/z) The acetone extracts of mangrove fruits were analyzed by direct infusion into the source by means of a syringe pump at a flow rate of 10 ml min⁻¹. ESI-MS fingerprints of the extracts and tandem mass spectra (ESI-MS/MS) were acquired in the positive and negative ion mode using a hybrid high-resolution and high-accuracy Micromass- Q-TOF mass spectrometer (microQTOF-QII 10330) Capillary and end plate voltages were set to 4600 V and -500 V, respectively with collision cell RF at 130 Vpp in positive mode, while in negative mode capillary and end plate voltages were set to 2500 V and -500 V, respectively, with collision cell RF at 130 Vpp. Nebulizer was set at 0.4 bar and at 180°C. The flow rate of the dry gas was fixed at 4.0 l/min in both modes of the mass spectrophotometer. The extracts were analyzed by direct insertion in positive and negative ion mode ESI-MS fingerprinting.

This method provides a sensitive and selective method for the identification of polar organic compounds with acidic sites, such as the phenolic

compounds found in wild edible fruits. Compounds of interest were then mass selected and their ESI-MS/MS were compared with data available in the literature.

Polyphenols absorb in the UV region⁸; for example, flavanols have λ_{\max} at 280 nm and hydroxycinnamic acid derivatives at 300-320 nm⁹. Characteristic UV-Vis absorbance spectra of acetone extract shows their occurrence in mangrove fruits. Five sample scans (λ ranging between 240-400 nm) are shown in a single graphical format (Fig 1)

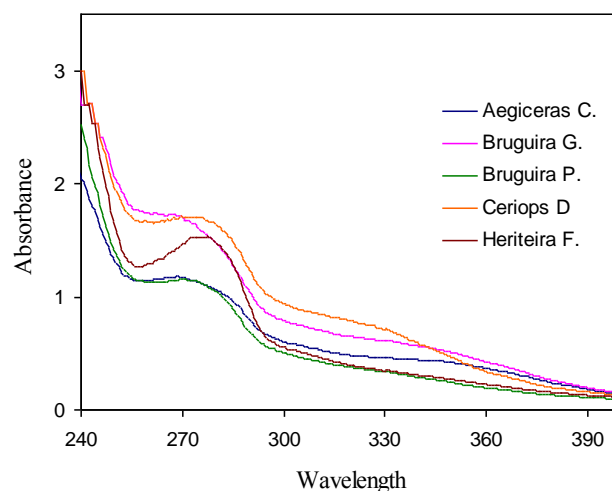


Fig 1. UV-Vis spectral scan of five main types of mangrove fruits

50 mg of the dry samples were weighed and homogenized with 5ml of 2.5 N HCl and placed over a boiling water bath for one and a half hours. After subsequent treatment of homogenized mixture with solid sodium carbonate, anthrone reagent was added and absorbance of the green colored solution was measured at 630 nm. Carbohydrate percentage was determined using extra pure glucose as standard.

Aqueous extract of 100 mg dry fruit samples were used for the determination of ascorbic acid. Sample extract containing 4% Oxalic acid was titrated against 2,6, dichlorophenol indophenol dye till the blue colour of the mixture changed to

pink at the end point. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the sample. A.R. quality ascorbic acid was used as the working standard (1mg/ml). Acidity of fruits was estimated by titrimetric method using phenolphthalein as indicator and expressed in percentage of citric acid.

For the determination of ash content, 1g of the sample was taken and it was placed in a silica crucible which was previously weighed very accurately. Next, the weighed crucible was subjected to heating at 550°C in a furnace for three hours. The sample was given ample time to cool down and it was weighed again. From the difference in the final and the initial weights of the crucible, the ash percentage was calculated.

2 g of the wet samples were taken in flat dishes and kept for 48 hours in hot air oven at 100-110 °C. Moisture content¹⁰ in fruits were determined from the weight loss. For the determination of carbohydrate, 1 g of the dried sample was taken in a 500 ml conical flask. 10 ml mixture of 1 N potassium dichromate and 20 ml of sulphuric acid was added to the sample. The mixture was shaken followed by the addition of 10 ml H₃PO₄ and 1 ml of diphenylamine indicator. The mixture was titrated using 0.4 N ferrous ammonium sulphate solutions till the blue colour of the mixture changed to green at the end point. The same experiment was repeated by adding standard glucose solution to the mixture in order to calculate glucose recovery.

Total phosphorous and nitrogen were examined by perdisulphate oxidation method. After extraction with potassium perxydisulphate, inorganic N and P content of the fruit samples were determined by spectrophotometric method¹¹. Total protein was computed multiplying nitrogen concentration by 6.25¹². The lipid was extracted from the fruit using alcoholic KOH and the extract was treated with cationic dye (pinacyanol). The coloured complex thus formed was extracted into bromobenzene and the lipid content in the extract was measured spectrophotometrically¹³.

Nutritive value of each fruit samples were determined by multiplying the value obtained for protein, fat and available carbohydrate by 4, 9, 4, respectively¹⁴. Nutritive value (kcal/100g) = (protein x 4) + (fat x 9) + (carbohydrate x 4)

Mean and standard deviation (SD) of three replicates of each sample were calculated and to assess the difference between the means of the variables student's t-test was performed. Statistical analyses were done by MINITAB (version 13.1).

Results and Discussion

Analysis of the carbohydrate content reveals that *Bruguiera paviflora* contains the highest percentage of carbohydrate (62.95±1.90) followed by *Ceriops decandra*. *Heriteira fomes*, contains lowest percentage of carbohydrate (27.25 ± 1.90). The percentage of phenolic compound is much higher in *Bruguiera gymnorrhiza* (12.35±0.65) than in *Ceriops decandra* (1.71±0.74). *Heriteira fomes* and *Bruguiera parviflora* exhibit almost comparable phenolic content (9.72±0.85, 11.67±0.95 respectively). Following phenolic compounds (Fig 2) in these two species were characterized by mass spectroscopy.

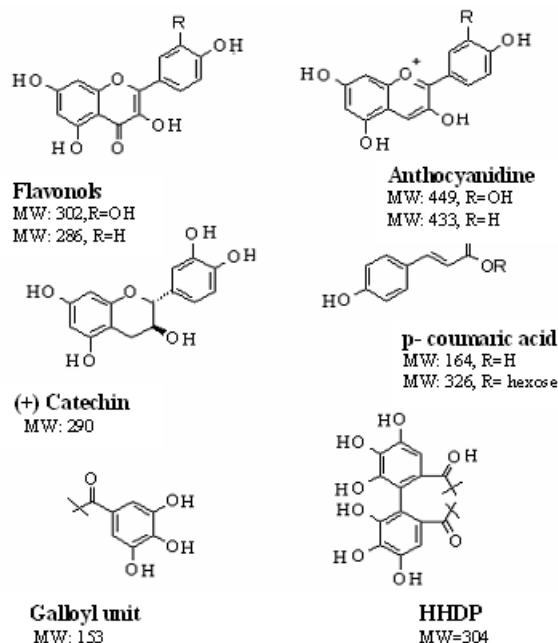


Fig 2: Structures of selected flavonols, anthocyanidins, (+)-catechin, p-coumaric acid, and HHDP

The percentage of both ash and moisture are high (3.52 ± 0.24 , 34.94 ± 2.05 respectively) in *Aegiceras corniculatum* fruit relative to other species. The concentration of ascorbic acid (mg/100g) is high in *Aegiceras corniculatum* (0.032 ± 0.002) and *Heriteira fomes* (0.026 ± 0.001) fruits, reflecting their antioxidant potentiality. *Heriteira fomes* exhibits the maximum protein percentage (45.48 ± 0.38) and *Ceriops decandra* contains minimum protein percentage (1.20 ± 0.01). Acidity (expressed in percentage of citric acid) is minimum in *Ceriops decandra* (0.128) is maximum in *Aegiceras*

corniculatum (0.395). Percentage of lipid content ranges between 1.75 and 4.31 with maximum in *Ceriops decandra* and minimum in *Bruguiera parviflora*. The variation of organic carbon is not significant in mangrove fruit samples. The percentage of organic carbon is maximum in *Aegiceras corniculatum* fruits (36.6) and minimum in *Ceriops decandra* (31.96%). The variation of total nitrogen is similar with total phosphorus. Total nitrogen and phosphorus are maximum in *Heriteira fomes* (7.27 ± 0.06 , 0.93 ± 0.011) and minimum in *Ceriops decandra* (0.19 ± 0.01 , 0.30 ± 0.005) (Table 1).

Table1. Chemical characterization of five mangrove fruits (mean \pm deviation (SD) in % dry weight and nutritive value in kcal/100g

| Fruits | Local name | Moisture content (%) | Ascorbic acid (mg/100 g) | Citric acid% | Ash (%) | Carbohydrate (%) | Protein (%) | Nutritive value | | | | Total N (%) | Total P (%) |
|-------------------------------|------------|----------------------|--------------------------|--------------|-----------------|------------------|------------------|-----------------|--------------|------------------|------------------|-----------------|------------------|
| | | | | | | | | Lipid (%) | (kcal/100 g) | Phenol (%) | Org C (%) | | |
| <i>Heriteira fomes</i> | Sundari | 22 \pm 2.23 | 0.026 \pm 0.001 | 0.235 | 3.15 \pm 0.04 | 27.25 \pm 1.90 | 45.48 \pm 0.38 | 2.12 \pm 0.52 | 309.99 | 9.72 \pm 0.85 | 34.74 \pm 3.25 | 7.27 \pm 0.06 | 0.93 \pm 0.011 |
| <i>Bruguiera parviflora</i> | Bokul | 20 \pm 3.01 | 0.013 \pm 0.003 | 0.271 | 2.65 \pm 0.95 | 62.95 \pm 1.39 | 15.65 \pm 0.22 | 1.75 \pm 0.75 | 330.16 | 11.67 \pm 0.95 | 34.27 \pm 4.10 | 2.50 \pm 0.05 | 0.70 \pm 0.008 |
| <i>Ceriops decandra</i> | Goran | 32.86 \pm 1.85 | 0.019 \pm 0.001 | 0.128 | 2.94 \pm 0.08 | 59.55 \pm 4.80 | 1.20 \pm 0.01 | 4.31 \pm 0.21 | 281.79 | 1.71 \pm 0.74 | 31.96 \pm 5.24 | 0.19 \pm 0.01 | 0.30 \pm 0.005 |
| <i>Aegiceras corniculatum</i> | Khalsi | 34.94 \pm 2.05 | 0.032 \pm 0.006 | 0.395 | 3.52 \pm 0.24 | 49.29 \pm 6.39 | 8.90 \pm 0.16 | 4.29 \pm 0.09 | 271.38 | 7.36 \pm 0.98 | 36.59 \pm 2.86 | 1.42 \pm 0.02 | 0.63 \pm 0.008 |
| <i>Bruguiera gymnorrhiza</i> | Kakra | 32 \pm 4.21 | 0.013 \pm 0.002 | 0.275 | 2.87 \pm 0.12 | 53.23 \pm 4.21 | 8.78 \pm 0.21 | 3.12 \pm 0.42 | 276.13 | 12.35 \pm 0.65 | 32.12 \pm 3.21 | 1.40 \pm 0.02 | 0.63 \pm 0.009 |

The mangrove fruits have a mean molar C, N, P ratio of 172:7:1 which is consistent with the composition reported for mangrove wood (748:8:1,¹⁵). However, the ratio is different in *H. fomes* (97:17:1) which contains considerably high concentration of protein-N (7.27%) and phosphorus (0.93%).

Chemical analysis of five mangrove fruits reveals that nutritive value of *Bruguiera parviflora* is maximum (330.16 kcal/100g) while *Aegiceras corniculatum* (271.38 kcal/100g) shows minimum. High calorific value of the fruits could be attributed to high carbohydrate and protein content. Some researchers¹⁶ found calorific value of 343.26±9.75 kJ/100 g for yellow velvet leaf (*Limnocharis flava* L. Buchenau) which is edible in Malaysia. The calorific values reported for some wild edible fruits are also high, ranging between 366 and 395 kcal/100g in Meghalaya¹⁷.

In edible fruits the vitamin C varies between 0.006 and 0.072 mg/100 g^{4, 18}. In the present study, the concentrations of vitamin C in mangrove fruits are found to be 0.013 – 0.032 mg/100g, similar to the mangrove fruits reported for Sri Lanka¹⁹. Nutritional data of some popular commercial fruits^{20,21} were given in table 2 and student's t-test was applied between the commercial fruits and wild mangrove fruits. Results suggested that there is significant similarity in carbohydrate ($t = 12.84$, $p = 0.001$) and ash ($t = 8.08$, $p = 0.015$) percentages and difference in their protein ($t = 0.82$, $p = 0.473$) and lipid content ($t = 0.7$, $p = 0.535$).

The phenolic compounds in mangrove fruits are characterized using UV spectra, MS spectra and mass fragmentation²³. Flavanols show maximum absorbance at wavelengths between 270 and 290 nm⁸. Many phenolics could also

absorb in this wave length range. UV-Vis spectra could be used as an indicative tool for the characterization of C-ring, whereas the MS spectra could provide additional significant information²². An absorption maximum at 280 nm and a marginally weak peak at 320 nm observed for mangrove fruits (Fig 1) indicates the occurrence of Flavanols. However, many compounds could interfere in this region²¹.

The ESI-MS fingerprint allowed the tentative identification of some phenolic compounds in acetone extract of both *Heriteira fomes* and *Bruguiera parviflora* fruits. Flavanols have high response in MS and in this study catechin (Molecular weight, MW 290) is found to occur in both the mangrove species. Ionic fragmentation of catechin in *Heriteira fomes* shows the value of m/z at 290/293/246/226/203 (Fig 3a) and *Bruguiera parviflora*, m/z at 290/293/246

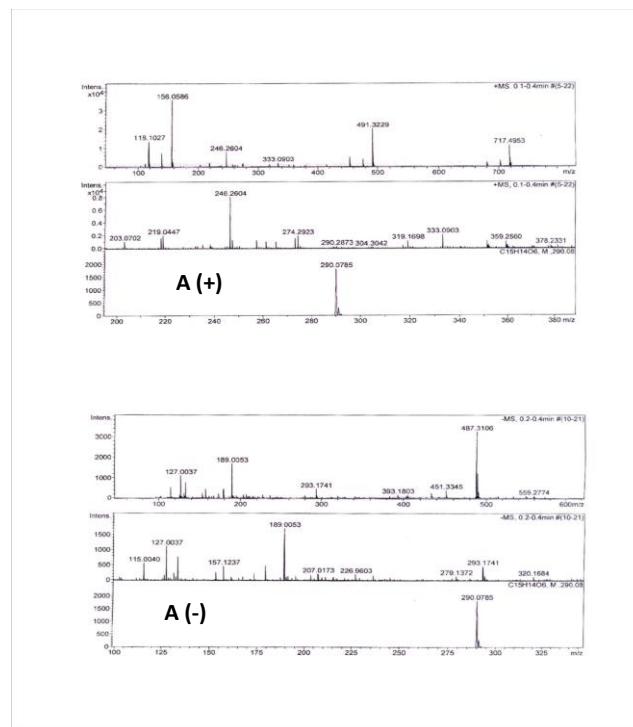


Fig 3 a: ESI-MS fingerprinting of acetone extract of *Heriteira fomes* (A) in both positive and negative mode.

| Commercial fruites | Ash(%) | Moisture(%) | Ascorbic acid(mg/100g) | Carbohydrate(%) | Protein(%) | Crude fat (%) | Nutritive value (kcal/100g) |
|--------------------|--------|-------------|------------------------|-----------------|------------|---------------|-----------------------------|
| Apple | 1.2 | 84.6 | 0.006 | 13.7 | 0.2 | 0.3 | 58 |
| Casheunut | | 5.9 | | 22.3 | 21.2 | 46.9 | 596 |
| Lichi | 1 | 84.1 | 0.072 | 13.6 | 1.1 | 0.2 | 61 |
| Mango ripe | 1.1 | 81 | 0.028 | 16.9 | 0.6 | 0.4 | 74 |
| Papaya ripe | 1.3 | 90.8 | 0.062 | 7.2 | 0.6 | 0.1 | 32 |

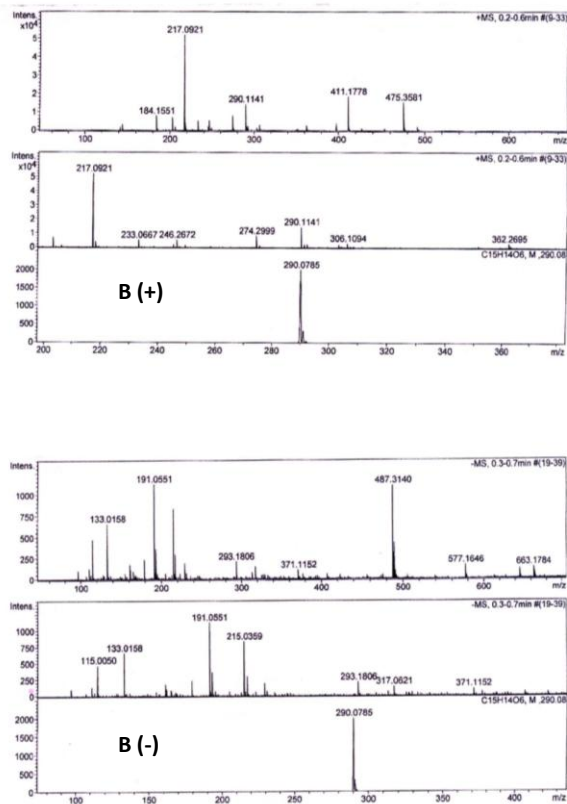


Fig 3 b: ESI-MS fingerprinting of acetone extract of *Bruguiera parviflora* (B) in both positive and negative mode

in positive and negative mode of the ESI-MS (Fig 3b) indicating the dominance of flavonol type catechin. Another flavonol type hydroxycinnamic acid derivative compound, p-coumarylhexose-4-hexoside (MW 488) is likely to occur in both the species at m/z 491/487/118/184. Identification of the isomers is possible if chromatographic separation technique is applied. There are some partly identified and non identified compounds in the wild mangrove fruits. Some partly identification of the phenolic compounds in both the fruit samples are reported here. The following compounds are identified: m/z 333 –mono-O-galloyl- β -D-glucopyranoside (MW 332), m/z 156/157- structure similar to cyaniding-3-glucoside (MW 450), m/z 451/190- quinine acid (191), m/z 306-ellagic acid derivatives (HHDP) (MW 475), m/z 577-proanthocyanidin B3 (C-4, 8-C) (MW 578).

The non identified fingerprinting with high intensity loads are found at m/z 189/127 in *Heriteira fomes* and m/z 663 in *Bruguiera parviflora*. Probably steroid type compounds represents in *Bruguiera parviflora* at m/z 215/217.

Conclusion

Mangrove fruits are consistent with the optimal value of carbohydrate, protein and vitamin-C mainly regarding culinary property and nutritional perspectives. ESI-MS study proves the presence of phenolic compounds responsible for their antioxidant nature

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